

The Chemical Nature of Holothurin, a Toxic Principle from the Sea-cucumber (Echinodermata: Holothurioidea)

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INTRODUCTION

A TOXIC principle in some Holothurioidea and its effects on fish and other small animals have been described in the literature (Saville-Kent, 1893; Frey, 1951). More recently, Nigrelli (1952) and Nigrelli & Zahl (1952) have reported on certain chemical and biological characteristics of a water soluble, heat stable substance isolated from the Bahamian¹ sea-cucumber, *Actinopyga agassizi* Selenka. This material, called Holothurin (Nigrelli, 1952), has been found to be toxic to a wide range of plants and animals, and also appears to have some anti-tumorous and other pharmacological properties².

Holothurin is a new variation on the theme of steroid compounds. It consists of several closely related aglycones or genins of steroid structure and a chain of several monosaccharides. It is soluble in water and produces a soapy foam in high dilution. Insofar as can be ascertained, this is the first known saponin of animal origin.

CHEMICAL STUDIES

The starting material of these experiments consists of sun-dried Cuvierian glands of *Actinopyga agassizi*. It forms pinkish flakes and is almost completely soluble in water. The material is first extracted in 95% ethanol and the minor fraction is discarded; it is then dissolved

in 50% ethanol, leaving behind cellular material and other debris. The resulting product, comprising 60% of the starting material, is neutral, practically free of nitrogen and exerts no reducing power for sugar reagents; it shows no absorption in the ultraviolet region of the spectrum and its specific rotation is about $[\alpha]_D^{25} = -19^\circ$. Upon hydrolysis with normal hydrochloric acid for three hours, it yields about 60% of its weight as a mixture of water soluble reducing sugars and 40% as a mixture of water insoluble aglycones.

The sugar solution, when chromatographed on paper with butanol-ethanol-water at pH=3.7, appears to consist of three different sugars, the fastest moving of which is probably rhamnose with $R_f=0.31$. The next one with $R_f=ca. 0.19$ gives pentose reactions and is presumably identical with xylose, while the slowest one with $R_f=0.14$ is glucose and may be removed by fermentation with yeast. Their quantities are in the approximate ratio of 2:1:1. This mixture of sugars is reminiscent of the carbohydrate portion of cardiac glycosides and other saponins of plant origin.

The water insoluble moiety resulting from acid hydrolysis melts, after softening at 200°, over a wide range from 230° to 250°. This mixture of aglycones (1.0 g.) may be fractionated by repeated crystallization from acetone, ether and other organic solvents, and yields four fractions in the following quantities: 130 mg. genin A, 70 mg. genin B, 90 mg. genin C, and 420 mg. fraction F. These fractions melt respectively at 256-260°, 235-240°, and 195-220°; fraction F is an oily substance, soluble in petroleic ether and partially soluble in aqueous alkali. The optical rotations are as follows: substance A $[\alpha]_D^{25} = -27^\circ$ (in ethanol), substance B $[\alpha]_D^{25} = -86^\circ$, and substance C $[\alpha]_D^{25} = -124^\circ$ (both in ethyl acetate). Fraction F presumably

¹We wish to thank Dr. C. M. Breder, Jr., Director of the Lerner Marine Laboratory of the American Museum of Natural History, Bimini, B.W.I., for the use of the laboratory facilities in collecting the Holothurin material used in these studies.

²We wish to thank Dr. Sophie Jakowska, College of Mount Saint Vincent, New York City, and Mr. Herman Baker, Department of Chemistry, The Mount Sinai Hospital, for their assistance in testing the pharmacological action of some of the products derived in these studies.

consists of fatty acids and esters, and its weak optical rotation of $[\alpha]_D^{25} = -8^\circ$ is probably due to contamination with some aglycone. Whether all of fraction F forms an intrinsic part of the molecule or is merely carried along by virtue of the detergency of the saponins is undecided. Substances A, B, C, as well as their acetyl derivatives, show ultraviolet absorption with $\lambda_{\max} = 244 \text{ m}\mu$ and an inflection at $238 \text{ m}\mu$. Extinction is highest, $E_{1\text{cm}}^{1\%} = 278$, for acetyl-A, and $E_{1\text{cm}}^{1\%} = 194$ for compound A itself. The acetyl derivative of fraction C shows a secondary maximum at $\lambda_{\max} = 300 \text{ m}\mu$ with $E_{1\text{cm}}^{1\%} = 19$.

On passing an aqueous solution of Holothurin prior to hydrolysis through a mixed bed ion-exchange resin column, all sodium and chloride ions may be removed. During this operation about 20% of the original solids, comprising electrolyte as well as much of the fatty material, remain on the column. The eluate is acidic; its optical rotation is $[\alpha]_D^{25} = -23^\circ$. The toxicity of the material seems to be diminished by this operation. This product has no reducing power and yields on acid hydrolysis the water soluble sugars and about 33% of its weight of the mixed neutral aglycones. Optical rotation $[\alpha]_D^{25} = -77^\circ$; elementary analysis: C, 72.27; H, 9.72; calculated for $\text{C}_{27}\text{H}_{44}\text{O}_5$ (average composition): C, 72.29; H, 9.88. M.p. $230\text{--}250^\circ$.

Fractionation of the aglycones yields a crystalline substance of m.p. $258\text{--}262^\circ$ and $[\alpha]_D^{25} = -19^\circ$, which is identical with substance A above. Elementary analysis: C, 69.87; H, 9.06; calculated for $\text{C}_{27}\text{H}_{42}\text{O}_6$: C, 70.10; H, 9.15. A second fraction, melting at $228\text{--}230^\circ$, parallels fraction B above in its properties. Elementary analysis: C, 70.90; H, 9.09 and inorganic residue 4.24%. These observations indicate that the derived aglycones are closely related one to another and consist of a tetracyclic steroid skeleton with two conjugated double bonds and five to six oxygen functions mostly of hydroxyl nature.

DISCUSSION

The carbohydrate components, presumably linked to a hydroxyl group on C_3 , are easily hydrolyzed by dilute acid. At the same time a

double bond (C:C or C:O) arises in conjugation with a previously present double bond. The wavelength of the resulting absorption maximum indicates that the two double bonds are situated in adjoining rings, e.g. in position $\text{C}_4:\text{C}_5$ (or $\text{C}_8:\text{C}_{14}$) and $\text{C}_6:\text{C}_7$. A highly substituted $\alpha:\beta$ -unsaturated ketonic structure cannot be excluded as an alternative. This unsaturated system may be independent of the glycosidic linkage, as it once arose on passage through the mixed bed column, where the glycoside portion of the molecule remains intact. The new double bond may be formed by saponification of an ester grouping in angular position and loss of the resulting free tertiary hydroxyl group by dehydration, or it may have arisen from the cleavage of an enol ether and concomitant migration of a double bond.

SUMMARY

Holothurin, the toxic principle in the Cuvierian organ of *Actinopyga agassizi*, a Bahamian sea-cucumber, is a steroid saponin. Acid hydrolysis yields a mixture of levorotatory genins with a conjugated system of two double bonds and three sugars—rhamnose, xylose and glucose. Some of the chemical features of the aglycones are discussed.

This is the first known steroid saponin of animal origin.

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